

Amendments to the SpecificationIn the Specification:

Please amend the specification as follows:

At page 1, please replace the paragraph at lines 6-17 with:

-- Related Applications

H₁ This application is a continuation application of U.S. Ser. No. 08/253,751, filed June 3, 1994, entitled "Methods for Selectively Stimulating Proliferation of T Cells" (now U.S. Pat. No. 5,858,358). The contents of the aforementioned application are hereby incorporated by reference. --

[At page 34, please replace the paragraph beginning at lines 34-38 and continuing onto page 35, lines 1-9 with:

-- Example 9: Long Term Growth of CD8⁺ T cells With Anti-CD3 and Monoclonal Antibody 2D8 ES5.2D8

H₂ Experiments were conducted to ~~determine~~ determine whether a population of CD8⁺ T cells could be preferentially expanded by stimulation with an anti-CD3 mAb and a monoclonal antibody 2D8 ES5.2D8. CD28⁺ T cells were obtained essentially as described in Example 1. To assay for CD8 expression, a primary anti-CD8 antibody and a labeled appropriate secondary antibody were used in FACS analysis to determine the percent positive cells. As shown in FIG. 17, at day 7 following stimulation of T cells with the anti-CD3 mAb G19-4sp and the mAb 2d8 ES5.2D8, the CD8⁺ fraction had increased from approximately 20% to over 40%. Another monoclonal antibody ER4.7G11 (referred to as 7G11) was also found to stimulate CD8⁺ T cells. This antibody was raised against recombinant human CTLA4 and has been deposited with the ATCC on Jun. 3, 1994 at Accession No. HB 11642. This result indicates that binding of either a

#2 distinct region of CTLA4 or of a cross-reactive cell surface protein selectively activates CD8⁺ T cells. - -

[At pages 35, please replace the paragraph at lines 11-25 with:

- - **Example 10: Defining the Epitope of the Monoclonal Antibody 2D8 ES5.2D8 and Cloning the CD9 Antigen**

H₃ To determine the epitope of the monoclonal antibody 2D8 ES5.2D8, epitope mapping was performed by phage display library (PDL) screening and was confirmed using synthetic peptides. A random 20 amino acid PDL was prepared by cloning a degenerate oligonucleotide into the fUSE5 vector (Scott, J. K. and Smith, G. P. (1990) Science 249:386-390) as described in Cwirla, S. E. et al. (1990) Proc. Natl. Acad. Sci. USA 87:6378-6382. The PDL was used to identify short peptides that specifically bound mAb 2D8 ES5.2D8 by a micropanning technique described in Jellis, C. L. et al. (1993) Gene 137:63-68. Individual phage clones were purified from the library by virtue of their affinity for immobilized mAb and the random peptide was identified by DNA sequencing. Briefly, mAb 2D8 ES5.2D8 was coated onto Nunc Maxisorp 96 well plates and incubated with 5 X 10¹⁰ phage representing 8 X 10⁶ different phage displaying random 20 amino acid peptides. Specifically bound phage were eluted, amplified, then incubated with the antibody a second time. After the third round, 7 phage were isolated, and DNA was prepared for sequencing. - -

[At pages 35, please replace the paragraph at lines 36-37 with:

H₄ - - In addition to CTLA4, a second antigen for mAb 2D8 ES5.2D8 was discovered using cDNA expression cloning. - -

[At pages 37, please replace the paragraph at lines 25-33 with:

-- B. Cloning Procedure

H5 In the cloning procedure, the cDNA expression library was introduced into MOP8 cells (ATCC No. CRL1709) using lipofectamine and the cells screened with mAb ~~2D8~~ ES5.2D8 to identify transfectants expressing a ~~2D8~~ ES5.2D8 ligand on their surface. In the first round of screening, thirty 100 mm dishes of 50% confluent COS cells were transfected with 0.05 µg /ml activated T cell library DNA using the DEAE-Dextran method (Seed, B. et al. (1987) Proc. Natl. Acad. Sci. USA 84:3365). The cells were trypsinized and re-plated after 24 hours. After 47 hours, the cells were detached by incubation in PBS/0.5 mM EDTA, pH 7.4/0.02% Na azide at 37 °C. for 30 min. --

[At pages ³⁷~~38~~, please replace the paragraph at lines ³⁴~~3-9~~ with:

H6 -- Detached cells were treated with 10 µg/ml mAb ~~2D8~~ ES5.2D8. Cells were incubated with the monoclonal antibody for 45 minutes at 4 °C. Cells were washed and distributed into panning dishes coated with affinity-purified goat anti-mouse IgG antibody and allowed to attach at room temperature. After 3 hours, the plates were gently washed twice with PBS/0.5 mM EDTA, pH 7.4/0.02% Na azide, 5% FCS and once with 0.15M NaCl, 0.01M Hepes, pH 7.4, 5% FCS. Unbound cells were thus removed and episomal DNA was recovered from the adherent panned cells by conventional techniques. --

[At pages 38, please replace the paragraph at lines 3-9 with:

H7 -- Episomal DNA was transformed into E. coli DH10B/P3. The plasmid DNA was re-introduced into MOP8 cells using lipofectamine and the cycle of expression and panning was repeated twice. Cells expressing a ~~2D8~~ ES5.2D8 ligand were selected by panning on dishes coated with goat anti-mouse IgG antibody. After the third round of

11.1
screening, plasmid DNA was prepared from individual colonies and transfected into MOP8 cells by the DEAE-Dextran method. Expression of a ~~2D8~~ ES5.2D8 ligand on transfected MOP8 cells was analyzed by indirect immunofluorescence with mAb ~~2D8~~ ES5.2D8 (See FIG. 18). --

[At pages 38, please replace the paragraph at lines 3-9 with:

-- BESTFIT analysis of the phage epitopes of mAb ~~2D8~~ ES5.2D8 to the amino acid sequence of CD9 revealed a close match:

H
8
T, 0380
G C W L L R E (phage 2D8#2, 4, 10; SEQ ID NO: 7)

G I W L R P D (phage 2D8#6; SEQ ID NO: 8)

G L W L R F D (CD9 sequence; SEQ ID NO: 9) --